Title page

Title: Hydrogen sulfide mediates the protection of dietary restriction against renal senescence in aged F344 rats

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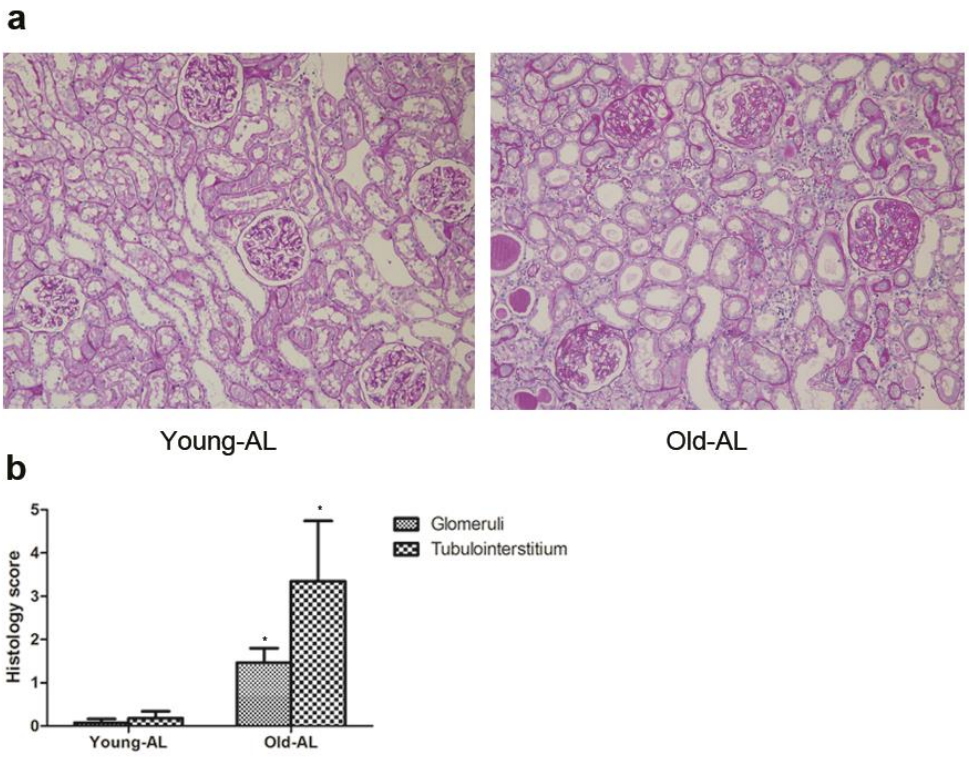
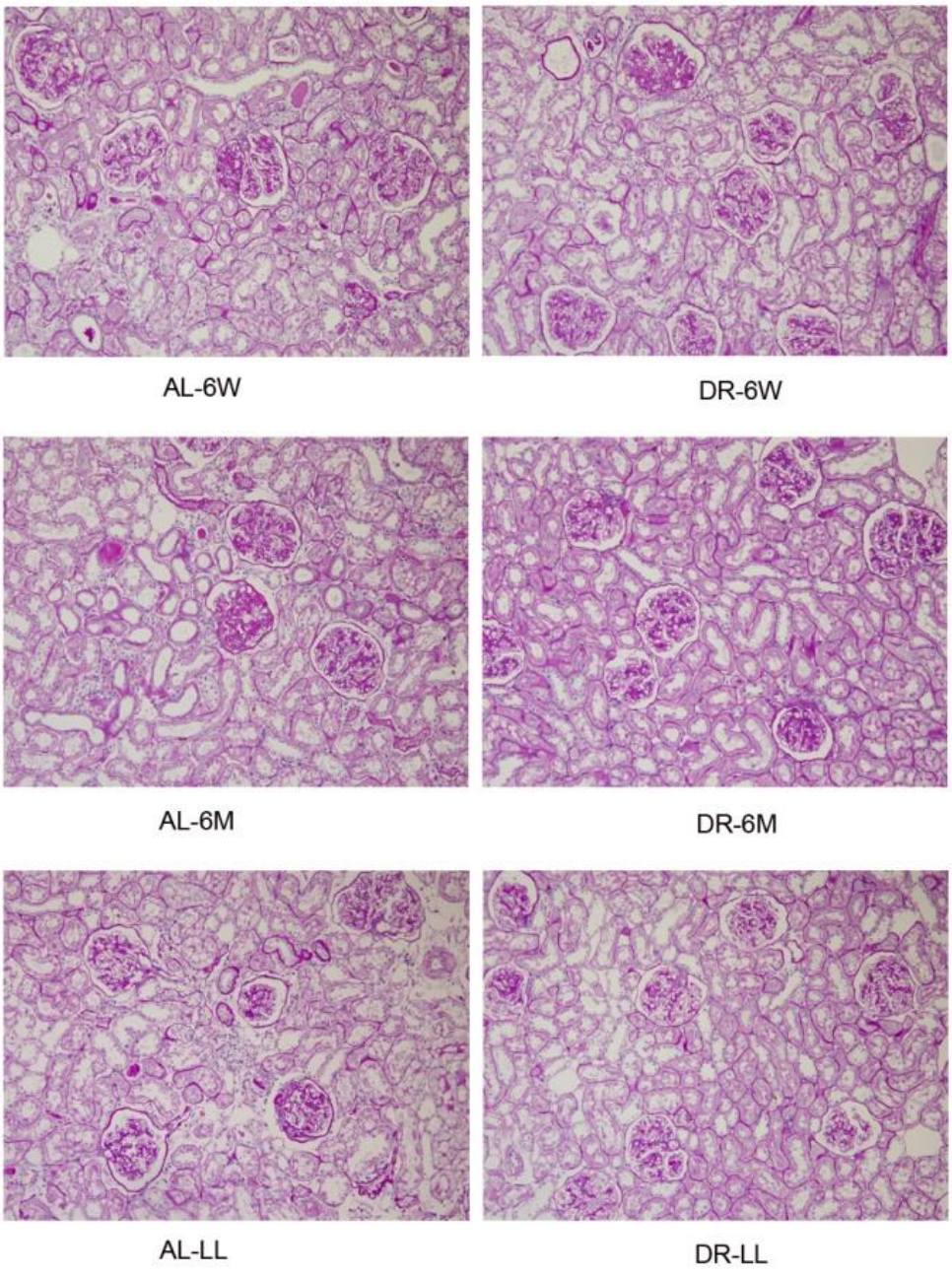


Fig.S1 Photomicrographs and histology scores of renal cortex in young and old rats. a For the Young-AL group, no abnormalities were found, while glomerular and tubulointerstitial lesions were severe in the Old-AL group. Renal tissue sections were stained using PAS staining. Magnification, ×200. b Compared with the Young-AL group, histology scores were significantly increased in the Old-AL group. Renal pathological grading by standard procedures from 20 random fields per rat. The data are presented as the mean ± SD (n = 5-8). *$p<0.05$ vs. the Young-AL group.

a


AL-6W
DR-6W
AL-6M
DR-6M
AL-LL
DR-LL

b

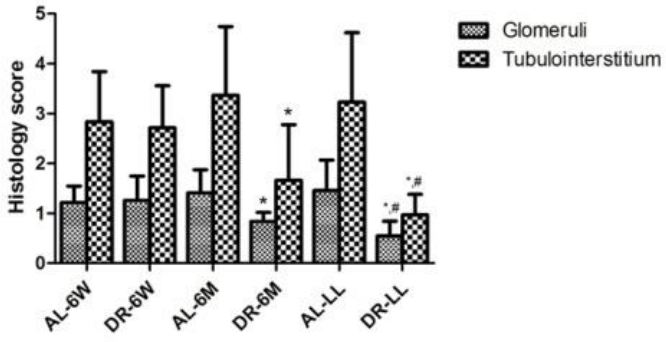
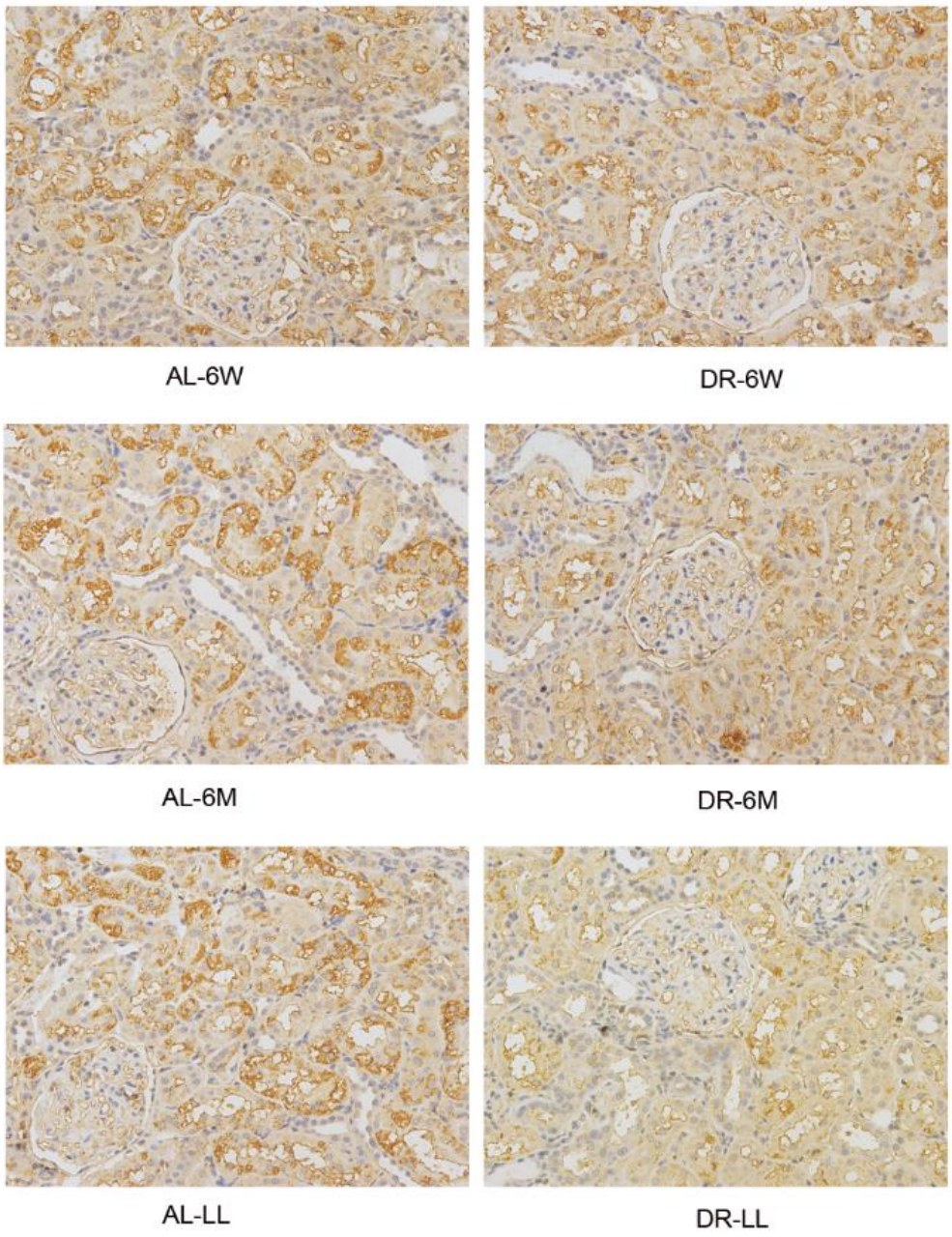

Histology score
0
1
2
3
4
5
Glomeruli
Tubulointerstitium
AL-6W
DR-6W
AL-6M
DR-6M
AL-LL
DR-LL
*
*
*#
*#

Fig. S2 Photomicrographs and histology scores of renal cortex in dietary restriction (DR) for different durations. a DR for 6-month (DR-6M) and life-long (DR-LL) alleviated age-related glomerular and tubulointerstitial lesions, but DR for 6-week (DR-6W) couldn't. Renal tissue sections were stained using PAS staining. Magnification, ×200. b Compared with the corresponding AL groups, histology scores were significantly decreased in DR-6M and DR-LL groups rather than in DR-6W. Renal pathological grading by standard procedures from 20 random fields per rat. The data are presented as the mean ± SD (n = 5-8). *$p<0.05$ vs. the corresponding AL. #$p<0.05$ vs. DR-6W.

a


AL-6W
DR-6W
AL-6M
DR-6M
AL-LL
DR-LL

b

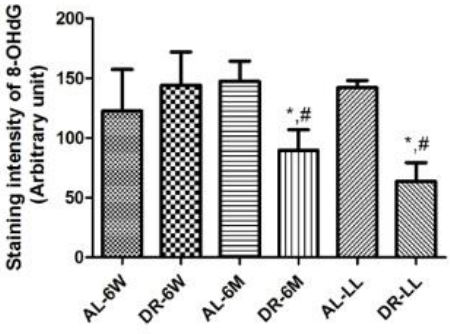

Staining intensity of 8-OHdG (Arbitrary unit)
200
150
100
50
0
*,#
*,#
AL-6W
DR-6W
AL-6M
DR-6M
AL-LL
DR-LL

Fig.S3 Detection of oxidatively modified DNA in old kidney tissues. a Immunohistochemistry staining results of 8-OHdG in renal tissues, magnification, ×400. It was mainly expressed in the renal tubular cytoplasm.b Quantitative analysis of 8-OHdG immunostaining intensity. Its intensity of immunohistochemical staining was analyzed in ten randomly selected mm^2 areas of renal cortex. The data are presented as the mean ± SD (n = 5-8). *$p<0.05$ vs. the corresponding AL. #$p<0.05$ vs. DR-6W.

Table S1 Metabolic indexes and kidney function parameters in the Young-AL and Old-AL groups.

	Young-AL (n=7)	Old-AL (n=19)
Body weight, g	236.3 ±12.28	684.92 ±48.69*
Kidney: Body weight, g g-1 ×100	0.73 ±0.06	0.57 ±0.13*
Serum urea nitrogen, mg dL-1	4.32 ±0.61	6.58 ±1.13*
Serum creatinine, μmol L-1	29.83 ±3.13	31.35 ±5.69
Triglycerides, mg dL-1	0.54 ±0.10	1.58 ±1.13*
Total cholesterol,mg dL-1	2.58 ±0.31	3.34 ±1.99
Serum glucose, mmol L-1	5.56 ±0.81	8.52 ±1.87*
Serum albumin, g L-1	37.5 ±6.23	34.91 ±3.23
Total protein, g L-1	62.20 ±9.31	67.45 ±4.82
Urine protein/urine creatinine ratio, mg mmol-1	98.69 ±7.79	450.84 ±50.72*

Data are mean ± SD

*P < 0.05 vs. Young-AL.

Table S2 Metabolic indexes and kidney function parameters in the aged rats on DR for different durations.

	AL-6W	DR-6W	AL-6M	DR-6M	AL-LL	DR-LL
Body weight, g	683.26±43.12	640.46±71.29	690.88±50.21	595±90.85*	680.63±50.05	540.78±47.91*,#
Kidney: Body weight, g g^{-1}×100	0.59±0.22	0.60±0.16	0.53±0.12	0.62±0.07*	0.58±0.09	0.69±0.1*,#
Serum urea nitrogen, mg dL^{-1}	6.44±1.24	6.00±0.88	6.38±1.27	5.39±0.93*	6.91±0.70	5.16±1.13*
Serum creatinine, μ mol L^{-1}	34.50±9.56	31.51±13.96	30.22±4.97	31.17±2.97	29.32±4.24	34.8±3.94
Triglycerides, mg dL^{-1}	1.33±0.93	1.38±0.95	1.68±0.49	1.02±0.29*	1.72±0.63	0.89±0.24*,#
Total cholesterol,mg dL^{-1}	2.97±2.31	3.39±1.03	3.65±0.83	3.44±0.67	3.41±0.93	2.85±0.70
Serum glucose, mmol L^{-1}	8.54±2.01	7.48±1.78	8.39±0.74	5.02±1.02*,#	8.63±1.01	5.51±0.98*,#
Serum albumin, g L^{-1}	33.91±5.38	33.56±6.12	34.67±3.13	36.05±2.19	36.14±3.37	34.41±3.72
Total protein, g L^{-1}	65.51±6.96	63.5±6.75	67.08±4.13	64.13±3.98	69.77±3.75	66.81±4.15
Urine protein/urine creatinine ratio, mg $mmol^{-1}$	441.8±43.11*	427.44±32.17	439.71±33.07*	362.04±39.69*,#	471.02±48.82	293.70±29.89*,#

Data are mean ± SD (n = 5–8).

*P < 0.05 vs. the corresponding AL.

#P < 0.05 vs. DR-6W.